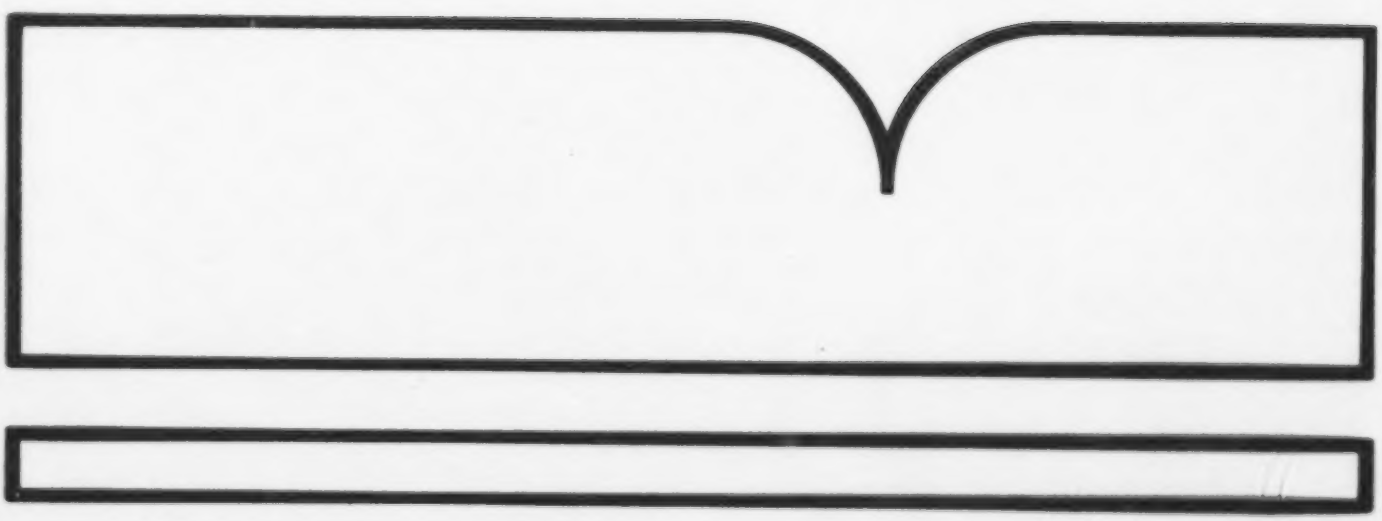
PB87-186789

DEF: Decision Document

(U.S.) Environmental Protection Agency
Washingotn, DC

Nov 81



U.S. Department of Commerce
National Technical Information Service
NTIS

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	PB87-186739

4. Title and Subtitle

DEF: DECISION DOCUMENT

5. Report Date 11/81

6.

7. Author(s)

EPA, OPP, REGISTRATION DIVISION

8. Performing Organization Rept. No. 540/09-87- 171

9. Performing Organization Name and Address

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
WASHINGTON, D.C. 20460

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.

(C)

(G)

12. Sponsoring Organization Name and Address

SAME AS #9

13. Type of Report & Period Covered

14. 500/01

15. Supplementary Notes

16. Abstract (Limit: 200 words)

This Position Document addresses the risks and benefits of pesticide products containing the subject active ingredient. The Agency has determined that the use of products containing the subject active ingredient may meet or exceed a risk criterion described in 40 CFR Part 154. Potential hazards will be examined further to determine the nature and extent of the risk, and considering the benefits of the subject active ingredient, whether such risks cause unreasonable adverse effects on the environment.

17. Document Analysis a. Descriptors

Pesticides, standards, regulations, manufacturing, chemistry, toxicology, residues, ecology, path of pollutants.

b. Identifiers/Open-Ended Terms

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

c. COSATI Field/Group

18. Availability Statement:	19. Security Class (This Report)	21. No. of Pages
Publicly Available	Unclassified	47
	20. Security Class (This Page) Unclassified	22. Price

540/09-87-171

DECISION DOCUMENT

DEF

Office of Pesticide and Toxic Substances

Office of Pesticide Programs

United States Environmental Protection Agency

Washington, D.C.

November 1981

TABLE OF CONTENTS

I. BACKGROUND	Page
A. Introduction	1
B. Legal Background and Regulatory History	2
C. Chemistry	4
D. Product Use	4
E. Dietary Exposure	6
1. Tolerances	6
2. Residue Monitoring	6
3. Dietary Exposure to the General Population	8
F. Applicator Exposure	9
1. Aerial Applicator Exposure	9
a. Inhalation Exposure	10
b. Dermal Exposure	11
2. Exposure During Ground Application of DEF	13

II. DEF AS A POTENTIAL RPAR CANDIDATE 14

A. Evidence Pertaining to Neurotoxicity 14

1. Animal Tests 15

2. Accident Reports From Field Use 25

3. Conclusion 26

B. Evidence Pertaining to Oncogenicity 30

C. Evidence Pertaining to Embryotoxic and Teratogenic Effects 31

III. SUMMARY OF CONCLUSIONS AND REGULATORY ACTIONS 35

Appendix A

Appendix B

BIBLIOGRAPHY

DEF DECISION DOCUMENT

I. BACKGROUND

A. INTRODUCTION

This document presents the Agency's decision not to initiate the Rebuttable Presumption Against Registration (RPAR) review of DEF. This decision is based upon the conclusion that even though laboratory animal testing indicates suggestive evidence of neurotoxic effects, actual field use of the chemical has not demonstrated unreasonable adverse effects to man or the environment. If the chemical were a neurotoxin of concern, the Agency would expect to see adverse effects in the field since neurotoxic effects are readily detectable within the approximate timeframe of chemical exposure. Moreover, the Agency has obtained agreements from registrants for label changes which specify protective clothing for applicators to further reduce exposure. Based on the available animal data, the Agency has calculated margins for various groups of applicators who wear protective clothing and found that adequate margins of safety exist.

The Agency's review of this chemical and the rationale for its conclusions are presented as follows. Section I contains legal background and regulatory history, chemistry, product use, dietary exposure and applicator exposure information. Section II evaluates the available evidence on DEF and reaches conclusions about the toxicological effects of concern. Section III summarizes the conclusions and the regulatory actions which the Agency will take as a result of this review.

B. Legal Background and Regulatory History

The Federal Insecticide, Fungicide, and Rodenticide Act, FIFRA (7 U.S.C. Section 136 et seq.), as amended, gives the Environmental Protection Agency (EPA or the Agency) the authority to regulate pesticide products. In order to obtain a registration for a pesticide under FIFRA, a manufacturer must demonstrate that the pesticide satisfies the statutory standard for registration. That standard requires (among other things) that the pesticide perform its intended function without causing "unreasonable adverse effects on the environment" [FIFRA Section (3)(c)(5)]. The term "unreasonable adverse effects" is defined as "any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide" [FIFRA, Section 2(bb)]. In effect, this standard requires a finding that the benefits of each use of the pesticide exceed the risks of use, when the pesticide is used in accordance with commonly recognized practices. The burden of proving that a pesticide satisfies the standard for registration is on the proponents of registration and continues as long as the registration remains in effect. Under Section 6 of FIFRA, the Administrator is required to deny or cancel the registration of a pesticide or modify the terms and conditions of registration whenever he determines that the pesticide does not satisfy the statutory standard for registration.

DEF was referred to the Special Pesticide Review Division (SPRD) as a candidate for the Rebuttable Presumption Against Registration (RPAR) process primarily because published data indicate that the herbicide induced neurotoxic effects in laboratory animals. The Agency also reviewed data on oncogenicity and reproductive effects as possible unreasonable adverse effects of DEF.

During the time period this chemical has been in review the 1978 amendments to the Federal Insecticide, Fungicide and Rodenticide Act were enacted. One major feature of these 1978 Amendments was Section (3)(c)(8), otherwise known as the Grassley-Allen amendment. Briefly, this amendment prohibits initiation of formal action (such as an RPAR review) to cancel, suspend or deny registration of a pesticide unless the action is based on a validated test or other significant evidence raising prudent concerns of unreasonable adverse risk to man or the environment. Defined, a "validated test" is one which is conducted and evaluated in a manner consistent with acceptable scientific procedure; "other significant evidence" is defined as that which relates to the uses of a pesticide and its adverse risk to man or the environment. It is intended that "other significant evidence" of adverse risks means factually significant information and is not to include evidence based on misuse of the pesticide, unless such use is generally recognized practice. In determining whether a particular pesticide raises "prudent concerns," the Agency examines the degree of toxicity of the pesticide, as well as the likelihood of human and environmental exposure. The Agency applies this approach to all its risk triggers, including oncogenicity and mutagenicity triggers which do not on their face take exposure into account. This approach allows the Agency to avoid the burdensome consequences of a RPAR procedure in those situations in which the uses of the pesticide at current levels of exposure do not appear to pose an unreasonable risk to man or the environment.

C. CHEMISTRY

The chemical name of DEF is S,S,S-Tributyl Phosphorotrithioate. Its molecular formula is $C_{12}H_{27}OPS_3$. Its structure is as follows:

$$(CH_3-CH_2-CH_2-CH_2S)_3P=O$$

DEF is a pale yellow, clear liquid having a molecular weight of 314.52. It boils at 150°C at 0.3 mm Hg, and melts at less than 25°C. The specific gravity is 1.057 at 20°C. DEF is soluble in aliphatic, aromatic, and chlorinated hydrocarbons and in alcohols. It is practically insoluble in water. It is relatively stable to heat and acids, and it hydrolyzes slowly under alkaline conditions producing the highly odorous n-butyl mercaptan.

D. Product Use

Two registrants currently hold Federal registrations for five products, all of which are liquid formulations. In 1961, technical DEF was registered. "DEF 6 Emulsifiable Defoliant" and "DEF 7" were registered as products in 1973 and 1975, respectively. The remaining two products were registered in 1972. At the present time, virtually all of the DEF in actual field use is DEF-6.

DEF[1/] is an organophosphate herbicide which was first registered in 1960 for cotton defoliation and this continues to be its only use. As a defoliant, DEF is applied in late summer and early fall as cotton crops reach maturity. Pre-harvest defoliation is commonly practiced for multiple purposes: to reduce boll rot and insect infestation, to increase the rate of boll opening, and to increase the yield and grade of cotton.

When DEF is applied at a rate of 1 to 1.5 pounds (active ingredient) per acre, 90 to 95 percent defoliation of cotton plants is usually achieved in 5 to 7 days. Aerial spray is the preferred mode of application (California, Texas, Arizona) except in the Mississippi Delta region, where--given the warm, humid climate--cotton plants grow taller than in other regions and where both ground and aerial applications are employed in order to reach cotton plants in their entirety.

[1/]Concurrently with DEF, the herbicide merphos (a chemical similar to DEF) has also been subject to Agency review. Like DEF, merphos became a candidate for the RPAR process primarily because published data indicated that merphos induced neurotoxic effects in laboratory animals. The conclusions drawn from the Agency's review of merphos are presented in a separate decision document.

E. Dietary Exposure

1. Tolerances

In 1969, Mobay Chemical Corporation (then Chemagro Corporation) petitioned for finite tolerances in the following commodities: cotton seeds, 4 ppm; meat, fat, and meat byproducts of cattle, goats, and sheep, 0.02 ppm (negligible residue); milk, 0.002 ppm (negligible residue) (40 CFR 180.272). The petition was granted. There is no separate tolerance established for cottonseed oil because the residues in cottonseed oil are covered by the residues in the raw commodity, cotton seeds. The Food and Drug Administration also established a tolerance for DEF at 6 ppm for cottonseed hulls for use as cattle feed (21 CFR 561.390).

2. Residue Monitoring

An investigation was conducted by Thomas and Harris (1965) with the dual purpose of 1) developing a gas chromatographic method for determining S,S,S-tributyl phosphorotrithioate in cottonseed and 2) applying this method in the analysis of cottonseed from cotton plants defoliated according to directions for use on the label for the product DEF. All treated samples received treatments of 2 pounds DEF active ingredient per acre. Samples were taken 1, 4, 8, and 11 days after treatment and analyzed by the colorimetric method as well as the gas chromatographic method. Both analytical methods yielded results indicating residues of DEF in or on cottonseed dissipate rapidly.

Approximately 90 percent of DEF residues dissipated within 4 days (from 2.8 ppm net residue on day 1 of the preharvest interval to 0.35 ppm on day 4, using the colorimetric method; from 5 ppm net residue on day 1 to 0.6 ppm on day 4, using the gas chromatographic method).

To measure the dissipation of DEF (and merphos) residues in cotton fields, the California Department of Food and Agriculture conducted field studies in 1975, in which residues in or on cotton plants were measured at varying intervals up to 9 days after application (Maddy, 1976). Five fields were studied, 3 of which were treated with DEF (Fields 1, 3, and 5), 2 with merphos (Fields 2 and 4). In Field 1 (near Bakersfield, Kern County), DEF was applied aerially at a rate of 1.13 pounds active ingredient per acre (spray volume: 12 gallons/acre). One hour after application, average total residue of DEF in or on cotton foliage was 161 ppm; 9 days after application, average total residue was 14.9 ppm. In field 3 (likewise near Bakersfield, Kern County), DEF was again applied aerially at a rate of 1.13 pounds active ingredient per acre (spray volume: 10 gallons/acre). One hour after application, average total residue of DEF was 214 ppm; 3 days after application, average total residue was 23.8 ppm. In field 5 (near Visalia, Tulare County), DEF was applied via ground spray at a rate of 2.25 pounds per acre (spray volume: 30 gallons/acre). One hour after application, average total residue of DEF was 286 ppm; 5 hours after application, average total residue was 185 ppm. The pattern of results observed in these field tests indicates that residues of DEF in or on cotton plants dissipate rapidly.

3. Dietary Exposure to the General Population

The Agency calculated dietary exposure for a 16-year old male weighing 70 kg by assuming that the residues of DEF (including DEF converted from merphos) are present at tolerance levels in meat (0.02 ppm), milk (0.002 ppm), and oil and oil products from cotton seeds in a 1.9 kg diet per day. While the Agency does not have data to indicate whether DEF is present at tolerance levels, the rapid dissipation of DEF would suggest that the tolerance assumption may overstate dietary exposure.[2/] The calculation showed that the maximum total daily intake of DEF is 1×10^{-4} mg/kg b.w./day (Datta, 1978). A four kilogram infant ingesting 770 ml of milk a day would have a total daily intake of 0.385×10^{-3} mg/kg/day.

2/The Agency has data indicating that DEF is not present even at the tolerance levels in milk, cottonseed oil, cottonseeds and cottonseed meal. The Food and Drug Administration (FDA) has monitored milk samples from 1972 through 1981, and found only trace amount of DEF (0.002 ppm) in one sample out of 5,000 samples tested. The FDA also monitors cottonseeds, cottonseed oil, and cottonseed meal for DEF residues which were found at levels much lower than the tolerance levels (cottonseeds, 0.06 ppm-Trace; cottonseed oil, 0.055-0.37 ppm; and cottonseed meal, 0.14 ppm-trace).

F. Applicator Exposure (See Appendix A for detailed calculations)

1. Aerial Applicator Exposure

The EPA and the State of California monitored exposure potential to flagmen, mixer/loaders and pilots during actual application of DEF on cotton fields in the San Joaquin Valley (Peoples et al., 1981). Applicators wore protective clothing.[3/] Protective clothing including socks, shirt, pants and a washable cap, clean cotton long-sleeved and long legged coverall and rubber boots were required daily and were worn by mixer-loaders and flagmen. Neoprene gloves were also worn by mixer-loaders. Pilots wore shoes and socks, a helmet and long-sleeved shirts and long-legged cloth pants which were changed daily. All applicators worked 7-hour work days.[4/] DEF-6 was aerially applied by certified applicators at the rate of 0.22 - 0.25 gallon (1.32 to 1.5 lb) active ingredient in 10 gallons of water per acre. Closed systems for mixing and loading were used.[5/]

[3/] Mobay Chemical Corporation, one of the registrants of DEF, has the following protective clothing requirements on their labels: "When handling, wear protective clothing (hat, long-sleeved shirt, long-legged trousers, shoes, socks) and synthetic or natural rubber gloves." Aceto Chemical has the following statement on their label: "Wear protective clothing, gloves, mask and goggles."

[4/] Seven hour work days are typical for professional certified applicators. Virtually all aerial application is performed by professional certified applicators.

[5/] Although the exposure estimates from this study may be lower due to the use of closed systems due to the study design, Agency scientists believe that the protective clothing specified for mixer-loaders will achieve the same exposure reduction without the addition of closed systems. While closed systems can reduce exposure if they are working perfectly, current technology tends to result in cases where closed system mechanical failures can increase exposure.

a. Inhalation Exposure

The amount of DEF inhaled was measured by placing a DuPont Constant Flow Sampler P-4000 pump on each worker. A total of 19 different breathing samples (7 for flaggers, 7 for pilots and 5 for mixer-loaders) were collected. Each air sample tube was monitored for one to seven hours. The final results are summarized in Table 1.

Table 1. Concentration of DEF Around Individual Workers During Aerial Application of DEF[1/]

Types of Workers	Concentration of DEF ug/m^3 Air		
	No. of Workers	Average	Range
Pilot	7	39.4	5.6-155.6
Flagger	7	72.9	18.2-191.2
Mixer/Loader	5	61.8	0.0-171.0

[1/]Based on a 7-hour period of measurement.

b. Dermal Exposure

Peoples et al. (1981) measured the DEF which fell on the patched area and the skin protected by cotton coveralls by attaching "patches" made of an outer layer of cloth and inner layer of gauze taped together. Each subject was fitted with patches on the arms, legs, front and back of neck and face. Hand washes were made to determine exposure to the hands, this value was added to the amount calculated for the rest of the body. The calculations for dermal exposures were based on the facts that the exposed workers wore long trousers and long-sleeved open-neck shirt and no gloves. The surface of face, back of neck, and the 'V' of chest were considered to be exposed areas. These facts were taken into account to obtain an average case exposure estimate. This estimate also assumes that 100% of the DEF measured from the bottom layer of patches worn on the clothing will reach and be absorbed into the skin. The total "average case" dermal exposure values for the individual groups of workers are shown in Table 2.

Table 2. Calculated Daily Dermal Exposures for Individual Workers During Aerial Application of DEF-6[1/]

Types of Workers	No. of Workers	Daily Dermal Average Exposure Values (mg/kg b.w./day) Average	Range
Pilot	7	0.14	0.07-0.25
Flagger	7	0.13	0.02-0.26
Mixer/Loader	6	0.13	0.06-0.20

[1/]Each individual worked 7 hours continuously during aerial application of DEF-6. Calculation of the exposure values assumed that the average male weighs 70 kg.

2. Exposure During Ground Application of DEF

Wilson et al. (1980) conducted a field exposure study with DEF using one applicator to estimate dermal and inhalation exposures. DEF was applied at the rate of 2.25 lbs. active ingredient in 25 gallons of water per acre. This is the maximum application rate listed on the label.

The air concentration was measured at 0.014 mg/m^3. The estimated inhalation exposure is 0.00252 mg/kg/day assuming a breathing rate of 1.8 m^3/hr for a 70 kg applicator working 7 hours a day. Although some farmer ground applicators may work up to 10 hours a day, the total exposure to complete the entire job is fixed (more hours per day result in fewer days). Thus, the 7 hour figure was used in the calculations.

The dermal exposure to DEF was measured by Wilson et al. (1980) by the analysis of patches worn on sleeves, shoulder, chest, thigh and legs of the applicator. An exposure of 117.5 mg/kg which is equivalent to 1.68 mg/kg/day is calculated for a 70 kg ground rig operator working seven hours a day and wearing protective clothing (long trousers and open neck long-sleeved shirt but no gloves or hat).[6/]

Since this study was carried out on only one applicator, the usefulness of this study to determine margin of safety is limited.

6/Although the current label requires a hat and gloves, the label in effect when this study was performed did not require them. The Agency did not adjust for a hat and gloves since discussions are ongoing with the registrants on this subject. It may be difficult to wear gloves while driving a rig and if the rig has a canopy, a hat may be unnecessary.

II. DEF AS A POTENTIAL RPAR CANDIDATE

DEF was referred to the EPA as an active ingredient suspected of meeting the 40 CFR 162.11 risk criteria warranting a rebuttable presumption. Delayed neurotoxicity as a potential hazard of the pesticide was the primary basis of concern, but oncogenicity and reproductive effects as possible adverse effects of DEF were also reviewed.

A. Evidence Pertaining to Neurotoxicity

40 CFR 162.11(a)(3)(ii)(B) provides that "a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) ... produces any ... chronic or delayed toxic effect [including neurotoxicity] in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account an ample margin of safety." A neurotoxic pesticide is defined in general terms as one which causes damage to the nervous system.[1/]

[1/] Certain organophosphate compounds cause a specific type of irreversible neurotoxicity commonly referred to as organophosphate induced delayed neurotoxicity. In this syndrome irreversible damage to motor nerves appears approximately two weeks after a single effective dose of the organophosphate. At this time the acute toxic effects of the compound have disappeared. The effect can also be produced by small daily doses of the compound which have no apparent immediate toxic effect. Histologically the condition is characterized by a degeneration of motor axons, particularly those serving the legs, followed by myelin degeneration. For reasons that are unknown at present, this toxicity cannot be demonstrated in the common experimental animals (rat, mouse, dog, etc.) and is best demonstrated in the adult chicken.

To determine whether DEF poses a neurotoxic risk warranting a rebuttable presumption, the Agency has reviewed and evaluated all currently available data.

1. Animal Tests (See Appendix B for tabled results of studies)

Indications that DEF might induce delayed neurotoxicity were first reported by Casida et al. (1963). In an experiment performed with two groups of White Leghorn hens, DEF was administered intraperitoneally at daily doses of 100 mg/kg. One group was treated for 10 days, the second group for 7 days. Ataxia was reported in the first group at 10 days and 18 days after the 10-day treatment commenced. In the second group, ataxia was observed on day 14 after the 7-day treatment commenced. The exact number of hens used in this experiment or the percentage exhibiting ataxia was not indicated, however, nor was a histopathological examination reported.

In 1964 Baron and Johnson reported that 26 of 27 White Leghorn hens given multiple intraperitoneal injections of DEF (50 mg/kg/day for 5, 10, or 15 days, or 100 mg/kg/day for 5, 10, or 15 days) showed clinical signs of delayed neurotoxicity as well as central and peripheral nervous system lesions. Histopathological examination (using the Marchi stain) reportedly indicated that spinal cord disruption might have occurred before the onset of clinical ataxia. In contrast to the results of intraperitoneal administration, Baron and Johnson (1964) reported that hens given oral doses of DEF (7 hens receiving 50 mg/kg/day for 5, 10 or 15 days; 2 hens receiving 100 mg/kg/day for 4 or 5 days) showed pathological lesions but no clinical signs of neurotoxicity. The daily oral doses of DEF of 100 mg/kg and above were not well tolerated and mortality was high; one hen survived administration of 150 mg/kg for 5 days and

showed both clinical and pathological signs of neurotoxicity. This oral study (50 mg and 100 mg dose levels), in which positive histopathology accompanied by negative clinical signs was observed, could not be repeated. In further experiments by Baron, he concluded that the original observation of positive histopathology but negative clinical signs was an artifact of the staining process (Baron, personal communication with Zendzian, 1979).

In screening tests for the production of paralytic effects in White Leghorn or Rhode Island red chicken hens, Gaines (1969) tested 9 carbamate and 30 organic phosphorous pesticides including DEF. All test chickens were given atropine sulfate in water solution by the oral route at a dosage of 15 mg/kg to protect them against the acute effect of each pesticide tested. Fifteen minutes later the pesticide was administered in peanut oil solution or suspension via subcutaneous injection under the wing. Test chickens were checked daily for signs of paralysis by placing them on the ground and observing their ability to walk. A single dose of DEF was then administered subcutaneously at 200 mg/kg to an unspecified number of hens. Hens (percentage unspecified) showed leg weakness beginning 14 to 28 days following injection with DEF and failed to recover completely by the end of the observation period of more than 30 days following injection.

Johnson (1970) conducted experiments with organophosphorous compounds, including DEF, and other inhibitors of brain "neurotoxic esterase" to examine the way in which delayed neurotoxicity develops in hens. For purposes of this study, a specially purified preparation of DEF (purity > 99 percent) was obtained from Chemagro Corporation. DEF was administered to 2 pairs of hens in single subcutaneous doses of 220 mg/kg and 1100 mg/kg, respectively. In each

pair of hens, one was observed for 3 weeks for signs of neurotoxic effects; the other was sacrificed after 24 hours and assays were performed on brain homogenate. Esterase activity was calculated as percentages of the same parameter measured in the brain of a normal bird assayed at the same time. Hens administered 220 mg/kg DEF did not show neurotoxic effects, and esterase activity was decreased only to 65 percent of normal. Hens administered 1100 mg/kg DEF showed neurotoxic effects, and esterase activity was decreased to 23 percent of normal.

Studies on the neurotoxicity of DEF via respiratory and dermal routes of exposure to White Leghorn hens were conducted by Thyssen and Schilde (1976a; 1976b) and submitted to the Agency by Mobay Chemical Corporation. Thyssen and Schilde (1976b) administered single dermal applications of technical grade DEF to hens at doses of 0.5, 1.0, and 2.0 ml/kg (502, 1,004, and 2,008 mg/kg, based on 1.057 as the specific gravity of DEF). As a positive control, TOCP was similarly administered at doses of 0.1, 0.2, 0.5, and 1.0 ml/kg. All applications were made to the featherless areas of the dorsal skin. Hens were observed for 28 days and histopathological examinations were performed on surviving hens at the end of the observation period. Neurotoxic effects (ataxia, paralysis, demyelination) were evident in hens dermally exposed to DEF at doses of 1.0 and 2.0 ml/kg, but no ataxia or nerve damage was reported at 0.5 ml/kg. In comparison, hens dermally exposed to TOCP showed neurotoxic effects at 0.2, 0.5, and 1.0 ml/kg, but not at 0.1 ml/kg.

In inhalation experiments, Thyssen and Schilde (1976a) reported neurotoxic effects when separate groups of hens were subject to a single 4-hour exposure, and five consecutive 4-hour exposures to DEF aerosols. The experiments were performed in a 15 m^3 inhalation chamber. Pure technical grade DEF (94 percent) was sprayed in concentrated form into the inhalation chamber by means of a paint spraygun fitted with a 0.5 mm nozzle, once at the start of each exposure period and then at 30-minute intervals throughout exposure. Each spraying lasted a maximum of 3 minutes.

Five hens per dose group were exposed to DEF for a single 4-hour period at doses of 391 mg, 878 mg, and 1585 mg per cubic meter of air. Neurotoxic symptoms (ataxia, paresis) were observed only at the high dose level (1585 mg/m^3) and neurotoxic effects were confirmed by histopathology. Ten hens per dose group were exposed for five 4-hour periods at doses of 62 mg, 145 mg, and 256 mg per cubic meter of air. Neurotoxic effects were observed at the 256 mg/m^3 dose level which were also revealed by histopathology. Slight delayed neurotoxic effects (uncoordinated leg movements) were seen at 145 mg/m^3, but no neurotoxic effects in hens subject to this dose level were detected by histopathology. No neurotoxic effects were detected in hens subject to the 62 mg/m^3 dose level. The method of exposure in these inhalation experiments was such that some DEF could have been absorbed through the skin or orally following preening.

Thyssen and Schilde (1978a) conducted another subacute inhalation study on chickens in which mature female chickens were exposed to DEF aerosol at concentrations of 8, 21 and 84 mg/m^3 for 6 hours per day, 5 days per week for three weeks. The only effects were observed at the highest dose — five of the

ten animals died and the remaining five developed signs of delayed neurotoxicity. Histological indications of neurotoxicity were only seen in one of the five affected animals. The authors concluded that DEF produced a neurotoxic effect only in the lethal concentration range of subacute inhalation studies.

In 1978, Thyssen and Schilde also performed a subacute dermal study on poultry (1978b). DEF was administered dermally to female adult chickens for three weeks, five days per week, for six hours per day. The dose levels were 0, 0.01, 0.03, 0.1, 0.3 and 1.0 ml/kg of body weight. Doses of 0.3 ml/kg and 1.0 ml/kg caused mortality of 50% and 100%, respectively. Obvious signs of delayed neurotoxicity were observed in the 0.1 and 0.3 ml/kg dose groups. The dose of 0.03 ml/kg (32 mg/kg) had no neurotoxic effects on the hens. The authors concluded that neurotoxic effects were seen only in the lethal dose range of 0.3 ml/kg (320 mg/kg) and at a dose 1/3 of the lethal dose range (i.e., 0.1 ml/kg). The Agency has accepted this study as valid.

The Agency contracted a study to be conducted by Dr. Abou-Donia of Duke University Medical Center (Abou-Donia, 1978; 1979a; 1979b) to further investigate neurotoxic potential of DEF and to determine a no observed effect level (NOEL). Abou-Donia conducted two separate experiments (1) single dose of DEF administered orally (2) subchronic (90-day) oral administration of technical DEF at lower doses to adult laying hens 19 months old and weighing 1.3 to 2.1 kg. Abou-Donia used a grading system of T_1, T_2, T_3, T_4, and paralysis in ascending order of toxicity to hens as follows: T_1, mild ataxia, as characterized by diminished leg movement, reluctance to walk, and a tendency to slide on the floor, or to fly; T_2, gross ataxia, characterized by disturbance in control of leg movement, with a change in gait; T_3,

severe ataxia, a stage in which legs sprawl out in front, and the hen is unable to stretch or lower its legs; and T_4, ataxia with near paralysis, marked by difficulty in moving the legs, inability to walk, and a tendency to sit down on the hocks most of the time. Paralysis is described as the inability to use the legs to stand, at which stage chickens usually show difficulty in breathing and swallowing, and some birds show a rapid, irregular head tremor. Three hens each were used for administration of Tri-O-cresyl phosphate (TOCP) (500 mg/kg), parathion (30 mg/kg), atropine sulfate (300 mg/kg), and empty gelatin capsules to act as positive, negative, atropine sulfate and gelatin capsule controls, respectively.

For the acute oral study, three atropinized hens each were given single doses of DEF in gelatin capsules at doses of 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 mg/kg of body weight, observed for 90 days and then sacrificed (Abou-Donia, 1978; 1979b). Histopathological examination was carried out on tissue samples of liver, brain, sciatic nerve and spinal cord which was negative for all doses. Clinical signs of ataxia were observed for all the doses but 50 mg/kg. By comparison, positive TOCP control hens produced ataxia and paralysis while parathion, atropine sulfate and gelatin capsules controls showed no signs of ataxia or other neurotoxic syndrome.

In Abou-Donia's subchronic oral study, nine groups of 5 chickens each were given daily doses in gelatin capsules of 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 or 20.0, 40.0 and 80.0 mg/kg for 90 days and then observed for additional 30 days (Abou-Donia, 1978; 1979a). The chickens were then sacrificed and histopathological samples were prepared. All the controls as described in single dose studies were also used in this study. Abou-donia observed T_1 and T_2 levels

of ataxia for all doses below 20.0 mg/kg doses except for the 0.1 mg/kg dose level. Only 20 mg/kg and the higher dose levels were histopathology positive and T_3 and T_4 levels of ataxia and paralysis were observed.

In an acute dermal study carried out by Abou-Donia, DEF was applied at 400 and 1,000 mg/kg single dose to the comb of the hens and observed for 30 days. T_1 and T_2 levels of ataxia were observed at the 400 mg/kg level, but histopathology was negative (Abou-Donia, 1979b). At 1000 mg/kg dose, the T_4 level of ataxia and paralysis were observed along with histopathological damage in spinal cord and sciatic nerve.

In a related subchronic dermal study, 20 and 40 mg/kg of daily doses of DEF were applied for 90 days on the comb of the hens and the hens were observed for an additional 30 days (Abou-Donia, 1979a). At the 20 mg/kg dose level, T_1 and T_2 levels of ataxia were found but the histopathology was negative; and at the 40 mg/kg daily dose, T_3 and T_4 levels of ataxia were observed and the histopathology was positive.

However, the work of Abou-Donia (1978) was audited by an EPA-FDA audit team (Beneke and Zendzian, 1980). The auditors concluded that although they found no serious problems with these studies, the results cannot be used to determine a definitive NOEL for delayed neurotoxicity. This conclusion was based primarily on the auditors opinions of Abou-Donia's scoring system. They reported:

"Dr. Abou-Donia's scoring system for signs of delayed neurotoxicity in the chicken utilize five degrees of impairment labeled T_1, T_2, T_3, T_4 and paralysis in ascending order of severity. The observations are subjective,

particularly in the T_1 and T_2 stages which are most important for determining a NOEL. Hens rated as T_3 or higher show abnormalities that can be readily demonstrated by placing the hen on the floor and observing its actions. The T_2 state may or may not be observable under the nonstandardized observing conditions utilized. Dr. Abou-Donia was unable to consistently demonstrate it in hens which he has rated T_2. The T_1 condition could be neither described by Dr. Abou-Donia nor demonstrated in hens which he had rated T_1" (Zendzian, 1980).

In contrast to the various positive reports discussed above, the following studies represent generally negative indications. A study was conducted for Chemagro Corporation by Harris Laboratories, Inc. (Harris, 1965b) in which adult Leghorn hens were maintained with DEF-treated feed for 30 days and thereafter observed and fed with untreated feed for another 30 days. Three groups of 6 birds each were administered DEF at dose levels of 100 parts per million (ppm) (12.5 mg/kg), 250 ppm (31.25 mg/kg), and 500 ppm (62.5 mg/kg) in feed. A fourth group of 6 birds was maintained on untreated feed during the 60 day test period. Harris (1965b) reported that none of the birds showed abnormal signs during the test period. Upon histopathological examination, one bird in each of the two lower dose groups showed scattered areas of demyelination of the sacral portion of the spinal column. No comparable changes were observed in the high dose group (500 ppm), although one bird showed focal liquification of the brain. Harris (1965b) proposed that since bacteria infection could cause changes similar to those observed, these histological changes might have been due to bacterial rather than chemical

toxicity. Because of this acknowledged possibility of bacterial infection, Harris Laboratories was asked to repeat the experiment described above, using the same test protocol (Harris, 1965a). Again, no abnormal clinical signs were reported in any of the test birds. Upon histopathological examination, no evidence of demyelination was found in any dose group. These Harris studies were submitted in the form of summaries and lacked details such as experimental procedures, data on individual animals and histopathological reports; therefore, no conclusion could be derived from these studies.

Two 30-day subacute feeding experiments were conducted by Thyssen et al. (1977) in which pure technical grade DEF (95 percent) was administered to adult White Leghorn hens at dietary concentrations of 0, 25, 50, and 100 ppm (0, 1.7, 3.5, and 6.3 mg/kg/day) in Experiment No. I; 0, 100, 200 and 400, ppm (0, 6.1, 10.9, and 18.5 mg/kg/day) in Experiment No. II. Test and control hens were 15 to 20 months old when the experiment began and weighed between 1.5 and 2.2 kg. A period of 14 days to allow hens to adapt to feed preceded the 30-day treatment period, after which those hens not immediately sacrificed for histopathological examination were observed for another 30 days. Depression of cholinesterase activity was noted in whole blood of treated hens, indicating that DEF was readily absorbed. Thyssen et al. (1977) reported that in neither experiment were clinical differences apparent between treated or control hens, either during the adaptation period, the treatment period, or the post-treatment observation period. No clinical signs of neurotoxic effects (ataxia, leg weakness, or paresis) were observed, and histopathological examinations showed no indications of delayed neurotoxicity.

Thyssen and Mohr (1976) reported negative results for delayed neurotoxicity when at an interval of 3 weeks, 300 mg/kg DEF (LD_{50} dose) was twice administered orally to White Leghorn hens antidoted with atropine sulfate (50 mg/kg) and PAM (100 mg/kg) formulated in physiological saline solution. Pure technical grade DEF was emulsified in polyethylene glycol 400 and administered to test hens by gavage. As positive controls, 5 hens were administered a single oral dose of TOCP, 375 mg/kg. Test and control hens were 15 to 18 months old and weighed between 1.4 and 2.0 kg. Of the 30 antidoted hens receiving the initial administration of 300 mg/kg DEF, 25 died. Twenty-one days following the first treatment, the 5 survivors were again antidoted, then treated with a second oral dose of 300 mg/kg DEF. All 5 hens survived this second treatment and were kept under observation for an additional 21 days. No clinical symptoms were observed in test hens after either administration of DEF. Among positive control hens treated with TOCP, signs of delayed neurotoxicity developed in 2 hens from day 8, and in the other 3 hens from day 12; the symptoms progressed from unsteadiness of gait through ataxia and lameness to total paresis of the legs. Following the 21-day observation period, positive controls and remaining test hens were sacrificed, and histopathological examinations were performed. No nerve damage was reported in hens treated with DEF. By contrast, positive controls treated with TOCP showed evidence of nerve fiber degenerations of varying intensity.

In a study by Wilson (1980) three featherless hens were placed next to an applicator driving a ground rig spraying DEF on cotton for seven hours a day for five consecutive days. These hens were observed for thirty days upon completion of the experiment for signs of neurotoxicity. No neurotoxic affects were found. Because there were no clinical signs of neurotoxicity, histopathology was not performed.

2. Accident Reports From Field Use

The Agency has also received reports of accidental exposures to DEF through its Pesticide Incident Monitoring System (PIMS). Most of these incidents come from California and none have come from the Mississippi delta region where ground use of DEF occurs. The Agency's file contains 30 incidents from 1966 to June, 1981 (EPA, 1981) or less than 2 per year on average. Out of 30 reports, 12 involved DEF alone, while the remaining reports concerned other chemicals as well. While the PIMS system is not 100% reporting, it provides an excellent indicator of the severity of a field problem for adverse effects which are detectable around the time of pesticide exposure. Pesticides which cause serious acute effects show high numbers of PIMS incident reports. For example, the PIMS report on paraquat, a cotton defoliant showed 318 incidents over a 13 year period or approximately 24 per year. PIMS reporting is also able to show reporting decreases after exposure reducing actions are taken (e.g. restricted use or protective clothing). The PIMS rate of 2 incidents per year for DEF is a low rate. Moreover, the effects reported were not severe. There were no reports of neurotoxic effects. Acute symptoms of pesticide poisoning (headache, nausea and skin rash) were observed but victims recovered fully within a few days. All these reported accidents were caused by accidental exposures due to drift, spillage, carelessness and/or not having proper protective clothing including long trousers, long sleeved-shirt and gloves.-

3. Conclusion

In summary, the available animal studies on the neurotoxicity of DEF have shown varied results (Summary of all studies are listed in Appendix B). While positive neurotoxic responses have been reported in animal tests employing intraperitoneal administration (Casida et al., 1963; Baron and Johnson, 1964), subcutaneous injection (Gaines, 1969; and Johnson, 1964), dermal and inhalation exposure (Thyssen and Schilde, 1976a; 1976b; 1978a; Thyssen and Mohr, 1978) and subchronic oral administration (Abou-Donia, 1978; 1979a and b), negative results have been reported in two animal feeding studies (Harris, 1965a and b, and Thyssen et al., 1977), in a gavage study (Thyssen and Mohr, 1976) and during actual application in the field (Wilson, 1980). Moreover while some of the data show that DEF causes a delayed neurotoxic effect, they do not clearly show whether the effects are reversible or not. Although the reversibility or the irreversibility of DEF is not a key factor in deciding whether to initiate an RPAR review, it would be an important part of the risk-benefit picture if existing margins of safety were not adequate.

The Agency has used the available animal data to calculate margins of safety. Studies appropriate to the human route of administration are used to calculate NOEL's but ranges are used where uncertainty surrounds the NOEL determination. Ranges are also used in calculating applicator exposure.

For the oral route, margins of safety (MOS) of 1,000-100,000 were calculated based on the assumption that a 70 kg adult male receives daily doses of DEF at tolerance levels (see Section I.E.3. above) and based on Abou-Donia's subchronic studies using 0.1 mg/kg/day or T_1 level (1.0 mg/kg/day) or T_2

level of ataxia (10.0 mg/kg/day) as NOEL's. MOS's of 260-26,000 were calculated for a 4 kg infant ingesting 770 gm of milk daily also based on Abou-Donia's study using 0.1 or 10.0 mg/kg/day. Abou-Donia's studies were used in this calculation because they were the most thoroughly conducted (90 day) oral studies which showed positive results. The NOEL ranges reflect the uncertainty in detecting clinical symptoms in the absence of positive histopathology.

For the inhalational route, MOS's were calculated for applicators exposed during aerial application (Peoples et al., 1981). Based on the average exposure values for applicators and based on the inhalation NOEL of 21.0 mg/m^3 (Thyssen and Schilde, 1978a), the MOS's were in the range of 288-538. The Thyssen study was used because its protocol most closely approximates the exposure scenario for professional applicators (e.g., 3 weeks of exposure per season).

MOS's for the dermal route were also based on the exposures measured for the applicators during aerial application (Peoples et al., 1981). Using the dermal NOEL of 32 mg/kg/day (Thyssen and Schilde, 1978b), MOS's for the average exposure levels were 229-246.

The Agency has calculated separate margins of safety for dermal and inhalation exposure since the animal studies reflect different NOELs. Normally, a single MOS would be developed. However, the exposure via inhalation is negligible when compared to the dermal exposure. For example, for ground applicators, Appendix A shows inhalation exposure is approximately 0.1% of dermal exposure. This same relationship would be true for aerial applicators. Therefore, the average range of the dermal margins of safety would accurately reflect the total margin of safety.

The acreage treated by ground applications is 5 to 10 percent of the total acreage treated with DEF. The ground applications are carried out for the rank cotton in the Mississippi delta region by the owner or the farm hand and confined to the single farm of the average size of 150 acres (Datta, 1979). Since an applicator can defoliate 50 acres a day, he does not get dermal and inhalational exposures of DEF for more than three days during an entire growing season. As shown in Appendix A, the total exposure is 5.05 mg/kg/yr, the NOEL is 480/mg/kg/yr (Thyssen and Schilde, 1978b), and the MOS is 95.

Most of the calculations of MOS's show significant safety margins. In addition, other significant factors are (1) whether neurotoxic effects have been observed in exposed applicators and (2) whether current label use precautions are adequate, or should be modified to improve safety margins for applicators. To date no human incident reports indicate that workers exposed to DEF during application operations have been affected by neurotoxic symptoms. The accident exposure reports show a low incidence of any effects and the low number of reported effects have been minor, reversible acute effects. It is reasonable to expect that the Agency would have been made aware of neurotoxic incidents had they occurred. First, neurotoxic effects such as paralysis occur a short time after initial exposure to DEF and are easily detectable. Second, EPA has an incident monitoring program; if neurotoxic effects caused by worker exposure to DEF occurred, such effects should have been recorded for at least a few such incidents. California, a State where a great quantity of DEF is used, has a well respected incident monitoring program. No such incidents have been recorded by California or any other State.

One of the two registrants for DEF, Mobay Chemical Company has recently modified their product labels to require protective clothing, including a long-sleeved shirt, long-legged trousers, shoes, socks, synthetic or natural rubber gloves and hat during handling. The other registrant, Aceto Chemical Company has agreed to modify their labels to include the above statement. The Agency believes these measures will reduce applicator exposure so as to ensure that the MOS's used in the Agency calculations occur in real world application situations.

B. Evidence Pertaining to Oncogenicity

40 CFR 162.11(a)(3)(ii)(A) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) "induces oncogenic effects in experimental mammalian species or in man as a result of oral, inhalation, or dermal exposure." The term "oncogenic" is defined in 40 CFR 162.3 (bb) as "the property of a substance or mixture of substances to produce benign or malignant tumor formation in living animals."

Current information concerning the oncogenic potential of DEF derives from a 2-year feeding study conducted by Root et al. (1967b) to determine the effects of lifetime exposure to rats. Four experimental groups of 48 weanling Sprague-Dawley rats each (24 males and 24 females) were fed diets containing 5, 25, 100, and 225 ppm DEF. Technical grade DEF containing 97.7 percent active ingredient was used to prepare the experimental diets. As a control, a fifth group of 24 males and 24 females was maintained on a diet containing 0 ppm DEF.

Histological analysis revealed that neoplasms were present, and neoplasia (pituitary adenoma, pheochromocytoma, fibroma, adenofibroma and adenocarcinoma) was more prevalent in female rats than in males. The analysis indicated, however, that the occurrence of neoplasms was not statistically significant nor was it dose-related.

Additional information indicated that at concentrations of 25 ppm DEF or less, the body weights of experimental animals did not differ significantly

from those of controls. In the group fed 100 ppm DEF, however, the growth rate of the male rats was reduced 12 percent during the last months of the study. At 250 ppm DEF, the average weight of female rats was reduced by 20 percent throughout the duration of the study. The age at death was also lowered for both sexes fed the 250 ppm DEF diet indicating maximum tolerated doses may have been fed. The study also indicated reduced cholinesterase activity at levels of 25 ppm DEF and above as compared to the control dose of 0 ppm DEF. Reduced cholinesterase activity was also observed at the 5 ppm level; however, it was considered insignificant. In the absence of any statistically significant indications of oncogenic effects, the Agency concludes that initiating the rebuttable presumption against DEF on the basis of oncogenic effects is not warranted.

C. Evidence Pertaining to Embryotoxic and Teratogenic Effects

40 CFR 162.11(a)(3)(ii)(B) provides that a "rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) ... produce any other chronic or delayed toxic effect (i.e., other than oncogenic or mutagenic effects) in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account an ample margin of safety." To determine whether DEF might pose an embryotoxic or teratogenic hazard warranting a rebuttable presumption, the Agency has reviewed currently available data to assess the potential of DEF to induce adverse perinatal effects. A summary of the Agency's findings follows.

Two reports by Russian authors on the embryotoxic effects of DEF in rats have been reviewed by the Agency. Gofmekler (1974) reported on 12 series of experiments intended to test the embryotropic effect of various chemical substances, including DEF, in connection with teratogenicity. Some of the chemicals used were alleged teratogens, and chemicals were tested in concentrations found in the atmospheric air in populated areas. An unspecified number of total experiments were conducted with 772 pregnant white rats and 6,200 embryos. Gofmekler (1974) reported that all chemicals tested including DEF elicited changes including the following: disturbance of reproductive functions (increase in intrauterine loss of fetus, drop in fertility, etc.); disruption of embryonic development (changes in weight indices and measurements of the embryos and their organs, subcutaneous hemorrhaging in the internal organs and cavity, disturbances in skeletal ossification); pathomorphological and histochemical changes in the uterus and placenta, and disruption in the balance of ascorbic acid in the female organism and in the embryo and placenta. No significant teratogenic abnormalities were observed, however.

Agency review of this report underscored a number of basic deficiencies, and the work of Gofmekler (1974) is of little use for purposes of evaluating the potential of DEF to induce reproductive effects. No precise information is given regarding the specific effects observed in terms of animals used, exposure levels, duration of treatment, or any other experimental parameters. Although an incomplete table indicates reduced fertility in DEF litters, and raises other general concerns, it is impossible to evaluate the effects reported on the basis of the information given (Chernoff, 1977).

In another study published originally in Russia and reviewed by the Agency in translation, Kasymosa (1975) reported on the embryotoxic effects of DEF when tested in female rats. The route of administration was apparently intragastric intubation, though difficulties in translation leave open the possibility that it was intraperitoneal injection. Six groups of 12 animals each were administered DEF at 0 (control), 0.95 mg/kg (1/250 LD_{50}) 2.45 mg/kg (1/100 LD_{50}), 12.5 mg/kg (1/20 LD_{50}), and 24.5 mg/kg (1/10 LD_{50}) body weight. In each group, 6 rats were treated on the third day of pregnancy, 6 on the tenth day. So that the character of fetal effects could be studied, some of the test animals were sacrificed on the tenth/eleventh day of pregnancy, some on the twentieth/twenty-first day, and some were allowed to give birth. Cholinesterase activity in the blood serum was also studied.

Kasymosa (1975) reported that a single administration of DEF on the third or tenth day of pregnancy reduced fertility at all dose levels tested. Cholinesterase activity was affected (reduced) only in the high dose group (24 mg/kg). Among rats administered DEF at 12.5 mg/kg and 24.5 mg/kg, total embryonic mortality was reported at 37.5 percent and 30.6 percent, respectively, as compared to 10.1 percent in controls, and the weight and physical dimensions of fetuses were reduced. Embryonic mortality in the groups administered 2.45 mg/kg or 0.95 mg/kg was not considered statistically significant, nor were the weight and size of fetuses affected.

Problems of translation, particularly with respect to the route of administration, render this Russian study of little use for purposes of evaluating the embryotoxic potential of DEF. The data would be relevant for

extrapolation only if the route of administration was in fact gastric intubation rather than intraperitoneal injection, and to elucidate the fetotoxic potential of DEF, it would be necessary to subject maternal animals to exposure for longer periods of time (Chernoff, 1977).

For purposes of assessing the potential of DEF to cause adverse perinatal effects, a study by Root et al. (1967a) is more useful to the Agency than the Russian studies discussed above. Root et al. (1967a) conducted a 3-generation breeding study in mice (Careworth Farms [CF1]) to test the reproductive effects of DEF in this species.

Four groups each containing 24 females and six males of proven fertility were fed experimental diets (0, 5, 25 and 100 ppm of DEF) for 30 days prior to mating. These experimental diets were continuous during pregnancy. All experimental diets were prepared using technical grade (97.7 percent active ingredient) DEF.

The work of Root et al. (1967a) was reviewed by the Agency (Burnam, 1978), and results indicated that continuous dietary exposure to DEF (up to 100 ppm) had no significant effect on litter size or on pre- or post-natal development of offspring. In addition, there was no indication that continuous dietary exposure (up to 100 ppm [12.8 mg/kg] DEF) resulted in adverse effects on fertility, gestation or lactation. Histological analysis did not reveal any specific or characteristic pathological findings that were compound-related.

Based on a review and evaluation of that evidence which is valid and interpretable, the Agency concludes that available data do not indicate that DEF poses a human hazard in terms of fetotoxic/teratogenic effects. A rebuttable presumption against DEF on the basis of adverse perinatal effects is therefore not warranted at this time.

III. SUMMARY OF CONCLUSIONS AND REGULATORY ACTIONS

The conclusions and recommendations arising from this review of DEF as an RPAR candidate conclude that the existing studies indicate that DEF does not produce unreasonable risk to man or environment. Oncogenicity and reproduction (teratology and embryotoxicity) studies do not indicate that hazards exist. Animal laboratory studies on neurotoxicity indicate a possible hazard but field studies do not support the existence of a hazard. Moreover, if the Agency assumes protective clothing is used, adequate margins of safety, based on animal laboratory data, exist for the general population and for applicators. Thus, applying the RPAR criteria and Section 3(c)(8) of FIFRA, it is not appropriate to consider DEF as a candidate for RPAR and DEF will be returned to Registration Division. No additional data are needed at this time. EPA will continue to work with registrants to clarify protective clothing label requirements.

APPENDIX A

MARGINS

Exposure Site	Exposure Range	NOEL	Margins
Inhalation (Aerial)	0.073 - 0.039 mg/m^3 of air	21.0 mg/m^3	$\frac{21}{0.073}$ = 288
	(See Table 1)	(Thyssen and Schilde, 1978a)	$\frac{21}{0.039}$ = 538 Margin 288 - 538
Dermal (Aerial)	0.14 - 0.13 mg/kg/day	32 mg/kg b.w. day	$\frac{32}{0.14}$ = 229
	(See Table 2)	(Thyssen and Schilde, 1978b)	$\frac{32}{0.13}$ = 246 Margin 229 - 246
Ground Application	0.00252 mg/kg/day Inhalation Exposure	32.0 mg/kg/day (Thyssen and Schilde, 1978b)	$\frac{480}{5.05}$ = 95 Margin is 95
Dermal	1.68 mg/kg/day Dermal Exposure	Total 15 applications (5 times a wk for 3 wks)	
Total	1.68 mg/kg/day Total Exposure for three days 1.68 x 3 = 5.05 mg/kg/yr	Total exposure for 3 wks 32 x 15 = 480 mg/kg/3 wks	
Dietary 70 kg (adult) 4 kg.(infant)	1 x 10^{-4}mg/kg/b.w. (Datta 1978) 0.385 X 10^{-3} mg/kg/b.w	0.1-10.0 mg/kg b.w./day (Abou-Donia 1979a)	$\frac{0.1 \text{ or } 10.0}{1 \times 10^{-4}}$ = 1000 - 100,000 $\frac{0.1 \text{ or } 10.0}{0.385 \times 10^{-3}}$ = 260 - 26,000

APPENDIX B

SUMMARY OF NEUROTOXICITY STUDIES REVIEWED
DEF

Report or Author	Mode of Administration	Dose Levels and Doses mg/kg	Results
Duke University Studies (Abou-Donia (DEF) (1978)	Oral	100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 (single doses)	Mild T_1 and T_2 levels of ataxia up to 800 mg/kg but regressed to T_1 during observation period. No histopathological damage at any dose level.
Ibid (DEF)	Oral	0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 (every day for 90 days)	No clinical effect at 0.1 dose. Histopathological damage only at 20.0 mg/kg and higher levels.
Baron and Johnson (DEF)	Intraperitoneal	100 mg/kg 5 daily doses	Positive ataxia. Positive damage in sciatic nerve and spinal cord.
(DEF)	Oral	50 mg/kg 5 daily doses	No ataxia observed but damage in sciatic nerve and spinal cord.
Casida Baron, Eto and Engler (DEF)	Intraperitoneal	100 mg/kg daily for 7 days and 100 mg/kg daily for 10 days.	Positive ataxia observed but histopathology is not reported.
Johnson (DEF)	Subcutaneous Injection	220 mg/kg 1100 mg/kg	35% depression in esterase. 77% depression in esterase.
Harris 16614 (DEF)	Oral in Feed	100 ppm(12.5 mg/kg) 250 ppm(31.25 mg/kg)	No ataxia or histopathological damage..
Harris 15948 (DEF)	Oral in Feed	100 ppm(12.5 mg/kg) 250 ppm(31.25 mg/kg) 500 ppm(62.5 mg/kg)	Demyelination at 100 and 250 ppm.

APPENDIX B

SUMMARY OF NEUROTOXICITY STUDIES REVIEWED

DEF (con't)

Report # or Author	Mode of Administration	Dose Levels and Doses mg/kg	Results
Johnson (DEF)	Subcutaneous Injection	220 mg/kg 1100 mg/kg	35% depression in esterase. 77% depression in esterase.
Harris 16614 (DEF)	Oral in Feed	100 ppm(12.5 mg/kg) 250 ppm(31.25 mg/kg)	No ataxia or histopathological damage..
Harris 15948 (DEF)	Oral in Feed	100 ppm(12.5 mg/kg) 250 ppm(31.25 mg/kg) 500 ppm(62.5 mg/kg)	Demyelination at 100 and 250 ppm.
Thyssen and Mohr (1976) 47932 (DEF)	Oral Gavage	300 mg/kg	Positive ataxia. No histopathological damage
Thyssen et al. (1977) 53640 (DEF)	Oral in Feed	0, 25(1.7mg/kg) 50(3.5 mg/kg) & 100(6.3 mg/kg)ppm in diet	Neither ataxia nor histopathological damage observed.
		0, 100(6.1mg/kg) 200(10.9mg/kg) & 400 ppm(18.5mg/kg) in diet	Neither ataxia nor histopathological damage observed.
Thyssen and Schilde (1976b) 49640 (DEF)	Dermal	0.5 mg/kg 1.0 mg/kg 2.0 mg/kg	No observable effect level 0.5 ml/kg. No ataxia and no histopathological damage.

APPENDIX B
SUMMARY OF NEUROTOXICITY STUDIES REVIEWED
DEF (con't)

Report # or Author	Mode of Administration	Dose Levels and Doses mg/kg	Results
Thyssen and Schilde (1976a) 50952 (DEF)	Inhalation	Single 4 hour exposure 5-4 hour exposure	No observable effect at 878 mg/m^3 for a single exposure and no observable effect at 62 mg/m^3 for multiple exposures.
Thyssen and Schilde (1978b) 67075 (DEF)	Dermal	1.0, 0.3, 0.1, 0.03 and 0.01 ml/kg 6 hours a day, 5 days a week for 3 weeks.	No observed effect level 0.03 ml/kg (32 mg/kg).
Abou-Donia (1979b)	Dermal	Daily doses of 20 and 40 mg/kg for 90 days on comb of hen.	Mild ataxia but negative histopathology at 20.0 mg/kg.
Abou-Donia (1979a)	Dermal	Single doses 100, 400, 1,000 on hen comb observed for 30 days.	Mild ataxia at 400 but negative histopathology. No abnormal results at 100 mg/kg; ataxia, paralysis, histopathological damage at 1000 mg/kg.
Thyssen and Schilde (1978a) 66480 (DEF)	Inhalation	Doses of 8, 21 and 84 mg/m^3 in air 6 hours a day-five days a week for 3 weeks.	No observed effect level is 21 mg/m^3

DEF BIBLIOGRAPHY

Abou-Donia, M.B. 1978. Study of the Low Level Effects of Organophosphorous Esters with Specific Reference to the Delayed Neurotoxic Syndrome. Quarterly Report #4. Unpublished, 33 pp. (Results subsequently published in Abou-Donia 1979a and b).

Abou-Donia, Mohamed B., Doyle G. Graham, Kamal M. Abdo, and A.A. Kameil. 1979a. Delayed Neurotoxic, Late Acute and Cholinergic Effects of S,S,S-Tributyl Phosphosotrithioate (DEF): Subchronic (90 days) Administration in Hens. Toxicology. 14:229-243. [Includes results reported by Abou-Donia 1978].

Abou-Donia, Mohamed B., Doyle G. Graham, Philip R. Timmons, and Barbara L. Reichert. 1979b. Delayed Neurotoxic and Late Acute Effects of S,S,S-Tributyl Phosphorotrithioate on the Hen: Effect of Route of Administration. Neurotoxicology. 1:425-447.[Includes results reported by Abou-Donia 1978].

Baron, R.L. and H. Johnson. 1964. Neurological Disruption Produced in Hens by Two Organophosphate Esters. Brit. J. Pharmacol. 23:295-304.

Beneke, Milton N. Jr., and Robert Zendzian. 1980. Summary of Findings [report of EPA/FDA audit of neurotoxicity experiments conducted by Abou-Donia (1978) under EPA contract 68-02-2452]. 11 pp.

Burnam, William L. May 22, 1978. Validation Report on Chemagro Report #21543 by Root et al. to Bipin Gandhi, Project Manager, SPRD. Unpublished.

Casida, John E., Ronald L. Baron, Morifusa Eto, and Judith L. Engel. 1963. Potentiation and Neurotoxicity Induced by Certain Organophosphates. Biochem. Pharmacol. 12:73-83.

Chernoff, Neil. 1977. Validation Reports on Papers by Kasymova and Gofmekler to Bipin Gandhi, Project Manager, SPRD. Unpublished.

Datta, P.R. 1979. Analysis of Human Exposure to DEF. Environmental Fate Branch, HED, OPP, EPA, Unpublished. 9pp.

Datta, P.R. 1978. Preliminary Human Exposure Analysis to DEF and Merphos. Environmental Fate Branch, HED, OPP, EPA. Unpublished. 30pp.

Environmental Protection Agency, 1981. Summary of Reported Pesticide Incidents Involving DEF. Pesticide Incident Monitoring System Report #459. 14 pp.

Gaines, Thomas B. 1969. Acute Toxicity of Pesticides. Tox. Appl. Pharmacol. 14:515-534.

Gofmekler, V.A. 1974. Embryotropic Action of Chemical Pollution of Atmospheric Air. (Russian Language Translation) gig. Sanit. 9:7-10.

Harris Laboratories, Inc. July 22, 1965a. Report on Demyelination Studies on Hens. Unpublished Chemagro Report #16614 (Proprietary). 3 pp.

Harris Laboratories, Inc. April 17, 1965b. Report on Demyelination Studies on Hens. Unpublished Chemagro Report #15948 (Proprietary). 10 pp.

Johnson, M.K. 1970. Organophosphorous and Other Inhibitors of Brain 'Neurotoxic Esterase' and the Development of Delayed Neurotoxicity in Hens. Biochem. J. 120:523-531.

Kasymova, R.A. 1975. Experimental Study of the Embryotoxic Effect of Butiphos (Russian Language Translation). Uz. Biol. Zh. 19(4):30-32.

Maddy, Keith T. 1976. Summary of Efforts in California to Resolve Alleged Health Problems Associated with Use of Cotton Defoliants. Agricultural Chemicals and Feed, Department of Food and Agriculture, State of California, Sacramento, California. Unpublished 114 pp.

Peoples, S.A., K. Maddy, P.R. Datta, L. Johnson, C. Smith, D. Conrad and C. Cooper. 1981. Monitoring of Potential Exposures of Mixer-Loaders, Pilots and Flaggers During Application of Tributyl Phosphorotrithioate (DEF) and Tributyl Phosphorotrithioite (Folex) to Cotton Fields in the San Joaquin Valley of California in 1979. pp 34. Unpublished.

Root, M., R. DiGiacomo, J. Moskauskas, and J. Doull. 1967a. Effect of DEF in the Diet on the Reproduction and Lactation of Mice. Toxicity Laboratory, University of Chicago. Chemagro Report #21543. (Proprietary). 7 pp.

Root, M., J. Moskauskas, and J. Doull. 1967b. Chronic Oral Toxicity of DEF to Male and Female Rats. Toxicity Laboratory. University of Chicago. Chemagro Report #20462, (Proprietary) 18 pp.

Thomas, R.F., and T.H. Harris. 1965. The Microcoulometric Determination of S,S,S-Tributyl Phosphorotrithioate in Cottonseed. J. Ag. Food Chem. 13(6):505-508.

Thyssen, J. and U. Mohr. 1976. DEF-Neurotoxicity Studies on Hens; Histopathological Findings. Chemagro Report #47932 (Proprietary).

Thyssen, J., and B. Schilde. 1976a. DEF-Neurotoxicity Studies on Hens (Inhalation Experiments). Chemagro Report #50952 (Proprietary).

Thyssen, J., and B Schilde. 1976b. DEF-Neurotoxicity Studies on dermally Treated Hens. Chemagro Report #49640 (Proprietary).

Thyssen, J., W. Wechsler, and B. Schilde. 1977. DEF-Neurotoxicity Studies on Hens (Thirty-Day Feeding Experiments). Chemagro Report #53640 (Proprietary).

Thyssen, J. and B. Schilde. 1978a. DEF-Neurotoxicity Effects on Poultry (Subacute Inhalation Study). Mobay Report #66480. (Proprietary).

Thyssen, J. and B. Schilde. 1978b. DEF - Studies on Subacute Dermal Neurotoxic Effects on Poultry. Mobay Report #67075. (Proprietary).

Wilson, B.W., C.M. Cisson, W.R. Randall, J.E. Woodrow, J.N. Seiber and J.B. Knaak. 1980. Organophosphate Risk Assessment: Field Testing of DEF with the Scaleless Chicken. Bull. Environm. Contam. Toxicol. 24:921-928.

Zendzian, Robert P. 1980. A memorandum to Jerry Moore, SPRD regarding Laboratory Audit, DEF and Merphos Studies of Dr. Abou-Donia. Received on October 30, 1980. Unpublished. 2 pp.

Zendzian, Robert P. 1979. Neurotoxic Review of DEF and Merphos. With a memo dated July 17, 1979, to Bipin Gandhi, Project Manager, SPRD. Unpublished. 14 pp.